

## THE FEEDING BEHAVIOR OF *PARANOPHRYS CARNIVORA* (CILIATA, PHILASTERIDAE)

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### ABSTRACT

The marine ciliate *Paranophrys carnivora*, isolated from the Mediterranean coast of Israel, was found to feed on a varied diet of bacteria, algae, and living and non-living tissues. *Chlorococcum* sp. and *Dunaliella parva*, the algal species on which *P. carnivora* grew best, did not elicit a chemosensory response; tissues and bacteria did. In experiments on stationary phase ciliates, betaine, choline, L-histidine, and trimethylamine oxide elicited a positive chemosensory response at concentrations as low as  $10^{-6}$  M to  $10^{-3}$  M.

### INTRODUCTION

Most ciliates feed on particulate matter consisting mainly of microorganisms (bacteria and algae) of a size appropriate to their buccal apparatus. The particles, whether suspended or settled, are collected via specialized cilia near the oral opening (Corliss, 1979).

In *Tetrahymena*, one of the ciliates most studied, particulate matter seems obligatory for feeding. An autoclaved 2% proteose peptone medium which supports a flourishing culture, loses this capability when the particles are removed by millipore filtration. Addition of inert particles lacking any nutritive value to the filtered medium restores its growth potential (Rasmussen and Kludt, 1970; Rasmussen and Modeweg-Hansen, 1973). Extensive experiments performed by Fenchel (1980a, b, c) with inert "latex" particles indicate that various ciliates select their food primarily by particle size.

When offered different combinations of algal species of the same size range, the ciliate *Favella ehrenbergii* showed a preference for one species, indicating that food selection is also based on factors other than size (Stoecker *et al.*, 1981). Selective feeding has been attributed to chemical stimuli in various other ciliates *e.g.*, *Nassula* (Poilvert, 1959), *Stentor coeruleus* (Tartar, 1961; Rapport *et al.*, 1972), and *Podophrya calkinsi* (Hull, 1961). In addition, particle movement affects the feeding behavior of ciliates (Karpenko *et al.*, 1977).

Most studies on the feeding behavior of ciliates focused on those feeding on microorganisms *e.g.*, *Paramecium*, *Tetrahymena* (Levandowsky and Hauser, 1978; Van Houten *et al.*, 1981, 1982; Antipa *et al.*, 1983; Levandowsky *et al.*, 1984; Leick and Hellung-Larsen, 1985; Hellung-Larsen *et al.*, 1986). Studies on ciliates that feed on tissues are scarce (Levandowsky and Hauser, 1978; Van Houten *et al.*, 1981). The marine ciliate *Paranophrys carnivora*, which was recently described, feeds on tissues of living or dead organisms (Czapik and Wilbert, 1986). The feeding behavior of this organism is described in this paper.

## MATERIALS AND METHODS

*Cultivation and morphology of Paranophrys carnivora*

*P. carnivora* was isolated from samples collected from the Mediterranean Sea at the coast of Dor, Israel. The samples were rich in various protozoans: Ciliata *e.g.*, *Euplotes*, and Flagellata, mostly autotrophic ones, including *Dunaliella*. Initial observations showed that *P. carnivora* fed on algae. They were also observed to gather in the vicinity of freshly injured invertebrates (crustaceans) and feed on their tissues.

Several *P. carnivora* clones were prepared and grown on *Dunaliella parva* as well as on a strain of the bacterium *Enterobacter aerogenes* which can grow at a salinity of 35%. No attempt was made to eliminate the original bacterial flora. The most successful clone was further cultured on *E. aerogenes* for growth curve and feeding behavior studies. These bacteria were grown on brain heart Agar (Difco) slants at 28°C, and then harvested in sterile water to give a suspension having an absorbance of 1.1–1.3 at 430 nm, as measured with a Bausch and Lomb "Spektronic 20" spectrophotometer. A 2-ml inoculum of a 3–4 day-old culture of *Paranophrys* and 0.4 ml of the bacterial suspension were added to a test tube (3 cm diameter, 20 cm length) containing 30 ml of sterile (autoclaved) 35‰ artificial seawater (Instant Ocean salts from Aquarium Systems, Mentor, Ohio, in filtered water, hereafter referred to as ASW). The culture was then incubated at 28°C in a temperature-controlled chamber. For growth curve studies, 0.5- or 1-ml samples were removed at various intervals from each of three cultures which had been inoculated simultaneously from the same source, and preserved in a 10% Bouin solution. All of the ciliates in each sample were counted in a glass chamber at 60× with a hand tally. Size determinations were made at 600× using an ocular micrometer.

The morphological description and identification was based on the same clone which was kept in 15 ml of ASW in covered glass vials at 20°C and fed every 14 days on 3 pin-head sized bits of either oligochaete or crustacean meat. Biometric measurements were made using a light microscope on ciliates stained by the protargol (Wilbert, 1975) and silver nitrate (Chatton and Lwoff, 1930) methods. For scanning electron microscopy, cells were fixed instantaneously by rapid addition of a large volume of 2.7% OsO<sub>4</sub> in ASW. After 10–15 minutes in fixative at room temperature, the cells were washed with 2% glutaraldehyde in ASW. After 10 minutes they were dehydrated in a graded ethanol series, dried by the critical point method, coated with gold-palladium, and viewed in a Joel 840 scanning electron microscope.

*Feeding and growth experiments with marine algae*

The species of marine algae whose names are given in Table II were cultivated in test tubes in a medium of ASW enriched with Walne solution (Walne, 1966) in a temperature-controlled room at 18°C and under continuous illumination. Young, flourishing week-old cultures were inoculated with *Paranophrys carnivora* and further incubated under continuous illumination at 25°C. The cultures were examined with a dissecting microscope at 40×—both initially and at various intervals during a week—to appraise the ciliate population growth. Samples were also examined under higher power of the light microscope, while alive and after they were killed with a 1% formalin solution. At 60×, the dimensions of the algae (length/width or diameter) were determined using an ocular micrometer. Ciliates were also examined for vacuoles containing algae.

*Behavior experiments*

*Capillary tube assay.* The amino acids tested were purchased from Sigma (L-leucine, L-isoleucine, L-proline, hydroxy-L-proline, L-arginine, D- and L-histidine,

L-cysteine); Nutritional Biochemical Corporation (DL-phenylalanine, DL-a-alanine, DL-serine, L-methionine, L-threonine, DL-asparagine); British Drug House, Ltd. (glycine, L-aspartic acid); Merck & Co., Inc. (L-tyrosine); Light & Co., Ltd. (L-cystine); Fluka (DL-valine); and CHR (L-tryptophan). Betaine hydrochloride, choline chloride, and trimethylamine oxide (TMAO) were also purchased from Sigma, and proteose peptone and brain heart infusion from Difco Laboratories.

The substances to be tested were dissolved in distilled water and the pH of the solution was adjusted, if necessary, to the pH of ASW (pH 8). Glass capillary tubes (Modulohm of Helver, Denmark) 5–8 mm long and 0.7–1.0 mm in external diameter, were filled with the test solution and dried. In a previous study with another species of the same family, *Parpostonia notatum*, many ciliates would enter a control capillary containing fresh medium even when the medium they were swimming in was only half-an-hour old (Kahn *et al.*, 1981). Therefore we modified the capillary assay by drying the test solutions and then, during the experiment, allowing the test substances to become dissolved in the same medium the ciliates were swimming in, rather than use fresh medium as a solute. Control capillary tubes were not filled with any chemicals. Tissue culture dishes, 35 × 10 mm (Falcon), were each filled with 2 ml of ciliates from the stationary phase of culture, which had been diluted with fresh ASW to a density ranging from 100 to 300 ciliates per ml. For each concentration and substance to be tested, a test and a control tube were placed in different halves of each experimental dish. When both test and control dry capillaries were immersed in the ciliate suspension at the start of each experiment, they became filled with the medium in which the ciliates were suspended. The test substances were dissolved within the experimental period (the substances found later to elicit a positive chemosensory response were further tested separately in a series of identical capillaries. These substances dissolved completely within a five-minute period). In each experimental run, a test and control capillary pair were tested in each of three dishes *i.e.*, in triplicate. Betaine, found earlier to elicit a chemosensory response from *Paranophrys carnivora* (mistakenly identified as *P. magna*; Kahan *et al.*, 1985), was used at a concentration of  $10^{-1}$  M (together with a control capillary) as a standard in each experimental run to verify the responsiveness of the ciliates. These betaine and control pairs were also run in triplicate. Using a dissecting microscope, the number of ciliates in each tube (up to about 100) was recorded with a hand tally at intervals during a 30-minute period. Levandowsky *et al.* (1984) preferred using flat capillaries to eliminate difficulties encountered in viewing *Tetrahymena* ciliates through cylindrical capillaries. We did not experience difficulty in counting moving *Paranophrys carnivora* in the cylindrical tubes. In the initial screening, most of the amino acids were tested at a concentration of  $10^{-1}$  M, with the exception of L-glutamic acid and L-tryptophan, at  $5 \times 10^{-2}$  M, and L-tyrosine, at  $2 \times 10^{-3}$  M. L-histidine was the only amino acid which elicited a positive response at least as strong as that of betaine. This amino acid, as well as betaine, choline, and trimethylamine oxide (TMAO), was further tested in at least four experimental runs at concentrations from  $10^{-6}$  or  $10^{-4}$  to  $10^{-1}$  M.

The chemosensory response was computed at each time interval as the ratio of the number of ciliates in the tube containing the test substance to the total number of ciliates in both the test and control tubes. Since statistical analysis (via *t*-tests) of the differences in response at different times during each half-hour experimental run showed no consistent effect of observation times, the index of chemosensory response for a given experimental run was defined as the maximum of the chemosensory responses at the time intervals measured. To adjust for variation in chemotactic responsiveness over the different days of the experimental runs, a relative index of chemotactic activity for a given substance at a given concentration, was defined as the ratio of



its chemotactic activity to the index of the standard (betaine, at a concentration of  $10^{-1} M$ ) for the same experimental run.

Both the index and relative index of chemosensory response of *P. carnivora*, for the various substances at different concentrations, were analyzed by two-way analysis of variance (Scheffé, 1959). Effects on the index due to substances or concentrations were analyzed using the S-method of multiple comparisons (Scheffé, 1959).

*Dialysis experiments.* Dialysis bags, 20 cm in length and 1.6 cm in diameter (Visking Tubing, The Scientific Instrument Center, Ltd.), were filled with 10 ml of either test solution (5% proteose peptone in ASW) or control (ASW alone). They were immersed in separate finger bowls each containing 150 ml of ciliate suspension at a density of 40 per ml. This was prepared by diluting a stationary phase culture with fresh ASW. To evaluate the behavioral effect, the tubing was first examined along its entire length—using the low magnification of the dissecting microscope—for the greatest congregation of ciliates. This section was further examined under  $40\times$  and the number of ciliates on both the test and control bags was compared at consecutive time intervals for up to 2 hours.

## RESULTS

### *The growth curve of Paranophrys carnivora and associated morphological changes*

The growth curve of *P. carnivora* fed on *Enterobacter aerogenes* at  $28^{\circ}C$  is shown in Figure 1. Figure 1 shows that the logarithmic growth phase continues for up to about 30 hours with a generation time of 7–8 hours. The stationary phase which follows is short, and after 48 hours there is a moderate decline in the number of ciliates. This phase continues until the experiments are terminated at the end of the fifth day. During the growth experiments the shape of the cell changed from ovoid (the “trophic” form, having a length to width ratio of 1.8 in the logarithmic phase) to more elongated (the “swimming” form, with a ratio of 2.2 or more in the stationary and decline phases). More pronounced differences between the two forms were obtained with cultures fed on either oligochaete or crustacean meat and, rarely, from cultures fed on algae. Scanning electron micrographs of the two forms from cultures fed on *Dunaliella parva* are shown in Figures 2 and 3. The biometric data presented in Table I are of silver stained specimens from cultures fed on oligochaete or crustacean meat, as are the light micrographs given in Figures 4 and 5. In Figure 5, note the marked appearance of the stained kinetosomes and the protrichocysts, another characteristic of the swimming form.

### *Feeding and growth experiments with marine algae*

Table II shows that *Paranophrys carnivora* ingested most of the algae offered. However, different results were obtained with the various algal species ingested. The best growth was obtained with *Chlorococcum* sp. and *Dunaliella parva*; no growth occurred with *Chlorella saccharophila* and *Dunaliella primolecta*. As might be expected, those algal species that were not ingested did not support good ciliate cultures.

### *Chemosensory response*

In the laboratory, *P. carnivora* fed on either *Enterobacter aerogenes*, various algae, or wounded *Artemia*, dead or alive, when each of these diets was offered individually. Differences in chemosensory responses were obtained when capillary tubes containing one of the diets was offered with a capillary containing no food (control), and the number of ciliates in each of the two tubes compared after 10 minutes. As shown in

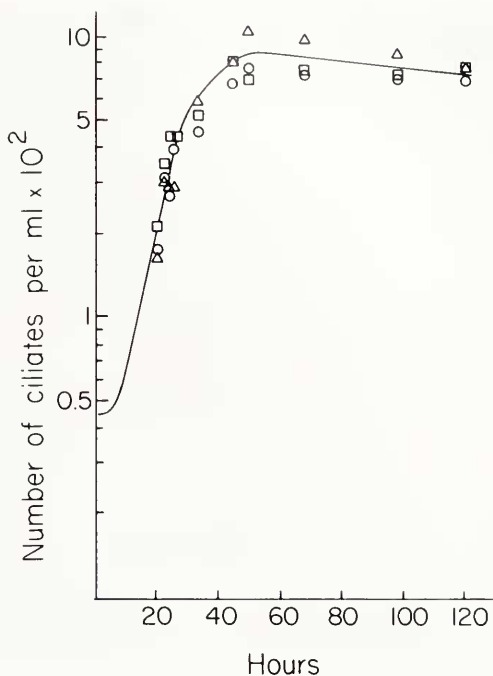
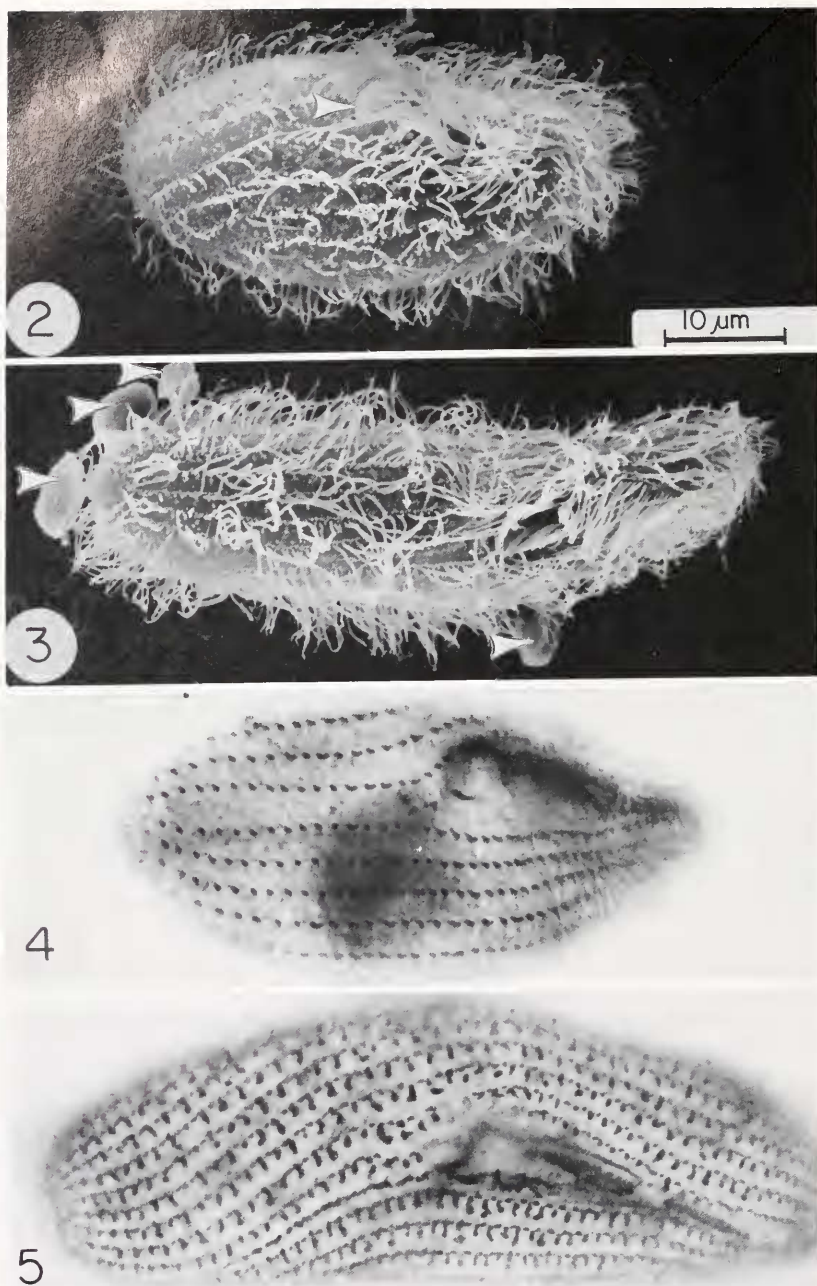


FIGURE 1. Growth curve of *Paranophrys carnivora*. Results are based on three replicate cultures.

Table III, *Artemia* homogenate and the *E. aerogenes* suspension elicited a positive chemosensory response, whereas the alga *Dunaliella parva* elicited none. These results were obtained with ciliates that had been previously cultivated on each of the diets indicated. When *Chlorococcum* sp. was offered instead of *D. parva*, the same results were obtained.

Since *Enterobacter* was routinely cultivated on brain heart agar, it was thought that its effect could have been due to the presence of some dissolved ingredients from the growth medium in the suspension. Indeed, brain heart infusion did elicit a positive chemosensory response in capillary experiments. To determine whether the bacteria themselves are effective, they were washed by centrifugation and offered to the ciliates in a capillary tube. After repeated rinsings in ASW, neither the bacterial pellet nor the supernatant gave a positive result. However, when the washed pellet was suspended in fresh ASW, incubated for up to 48 hours at 18°C, and then centrifuged again, the resulting supernatant elicited a chemosensory response. This indicates that washed bacteria excrete with time an effective substance or release such a factor upon disintegration.

Positive results were obtained with other microbiological growth media, such as proteose peptone and casein hydrolysate. In dialysis experiments with proteose peptone, *Paranophrys* was found to be attracted to those molecules which were able to pass through the cellophane membrane, e.g., amino acids. In further capillary tests to screen individual amino acids, only L-histidine, of the various amino acids tested, was as effective as betaine, a substance previously found to elicit a chemosensory response from *P. carnivora* (Kahan *et al.*, 1985). Choline and trimethylamine oxide (TMAO), compounds with a chemical structure similar to that of betaine, also elicited a positive response at least as strong as that of betaine. The four substances (beta-



FIGURES 2-5 are to the same scale and view the ventral side (note buccal cavity).

FIGURE 2. Scanning electron micrograph of trophic form of *Paranophrys carnivora* (arrowhead indicates part of an algal cell, *Dunaliella parva*, engulfed by the ciliate).

FIGURE 3. Scanning electron micrograph of swimming form of *P. carnivora* (arrowheads indicate algal cells from culture which have adhered to the ciliate).

FIGURE 4. Photomicrograph of silver-stained trophic form of *P. carnivora*.

FIGURE 5. Photomicrograph of silver-stained swimming form of *P. carnivora*.

TABLE I

*Cell dimensions of the trophic and swimming forms of Paranophrys carnivora (given in micrometers)*

Form	Length		Width			Distance from anterior pole to end of UM $\bar{x} \pm S.E.$
	Range	$\bar{x} \pm S.E.$	Range	$\bar{x} \pm S.E.$	Range	
Trophic	36-56 (17)	47.08 $\pm$ 1.3	18-35 (22)	25.18 $\pm$ 1.07	18-25 (17)	20.82 $\pm$ 1.17
Swimming	40-60 (18)	49.44 $\pm$ 1.47	13-22 (15)	16.67 $\pm$ 0.62	24-29 (15)	26.4 $\pm$ 0.52

Numbers in parenthesis indicate the number of observations.

ine, L-histidine, choline, and TMAO) were offered to *P. carnivora* at various concentrations; the results were analyzed as described previously. The index averaged over the experimental runs is shown in Figure 6 for each substance at the various concentrations. The average index of chemosensory response elicited by betaine, choline, and TMAO was significantly greater than 0.5, at the 5% level or more, at all the concentrations examined, and by L-histidine for concentrations of at least  $10^{-3}$  M (concentrations of  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  M were also tested, but the response was not significantly greater than 0.5).

Two-way analysis of variance of the index of chemosensory response showed significant effects due to material ( $P$ -value = 0.028) and concentration ( $P$ -value = 0.000). The means of the relative index of chemosensory response (the index, previously defined under Materials and Methods as "capillary tube assay," which adjusts for the level of response to the betaine standard during the same experimental run) exhibited the same behavior as the nonadjusted index, indicating that the differences in the index of chemosensory response represented in Figure 6 are not due to varying levels of overall chemosensory responsiveness (as measured by the response to the standard, betaine at  $10^{-1}$  M) on the different days of experimental runs. The analysis of variance is based on the nonadjusted chemosensory response, as opposed to the adjusted response, because the data on the former satisfied the required statistical assumptions on the error terms more closely (see Scheffé, 1959, p. 5).

Figure 6 suggests that choline, betaine, and TMAO are similar in the response they elicit from *P. carnivora*, the main differences in the chemosensory response index being due to different concentrations. This was confirmed for betaine and choline using the S-method (Scheffé, 1959) of multiple comparisons to compare effects on the chemosensory response index due to materials or concentrations. The average index for betaine and choline at the concentration of  $10^{-1}$  M was significantly different (at the 5% level) from the average at the  $10^{-6}$  M level; and the differences between the averages at the  $10^{-1}$  M and  $10^{-5}$  M levels, and at the  $10^{-2}$  M and  $10^{-6}$  M levels, were almost significant at the 10% level.

#### DISCUSSION

Although previously thought (Czapik and Wilbert, 1986) to feed on fresh and decomposing tissues, the present study establishes that *Paranophrys carnivora* can feed on a more varied diet. This diet includes algae and bacteria in addition to tissues. In this respect it seems to be closer in its dietary spectrum to *P. thompsoni* and *P. magna* than to the other species of the genus. *P. thompsoni* was reported to live on bacteria and heterotrophic flagellates which developed in hatched gelatinous egg



TABLE II

Growth on and ingestion of different algal species by *Paranophrys carnivora*

Algal class: Species	Source	Algal size <sup>a</sup>	Ingestion of algae	Ciliates' growth <sup>b</sup>
Bacillariophyceae				
<i>Amphora</i> sp. 12	J.L., CUNY <sup>c</sup>	35-40/2-4	no	+
<i>Phaeodactylum</i> <i>tricornutum</i>	CMBRDG CC <sup>d</sup>	20-30/2.5	yes	++
Chlorophyceae				
<i>Chlamydomonas</i> <i>provasoli</i>	J.L., CUNY	4-8	no	+
<i>Chlamydomonas</i> <i>hedleyi</i>	J.L., CUNY	5-10/3-9	yes	++
<i>Chlorella</i> <i>stigmatophora</i>	IOLR <sup>e</sup>	5-6/3-4	yes	+
<i>Chlorella</i> <i>saccharophila</i>	IOLR	3	yes	-
<i>Chlorococcum</i> sp.	J.L., CUNY	2-3	yes	++++
<i>Dunaliella</i> <i>primolecta</i>	CMBRDG CC	6-14/4-13	yes	-
<i>Dunaliella</i> sp. Strain C9AA	B.G., HU <sup>f</sup>	10-18/8-13	no	+
<i>Dunaliella</i> sp. Strain E1	B.G., HU <sup>f</sup>	14-18/8-10	no	+
<i>Dunaliella</i> sp. Strain 1644	B.G., HU <sup>f</sup>	11-21/8-15	no	+
<i>Dunaliella</i> sp. Strain L10	B.G., HU <sup>f</sup>	9-14/8-13	no	+
<i>Dunaliella</i> <i>tertiolecta</i>	B.G., HU <sup>f</sup>	8-12/4-8	yes	+++
<i>Dunaliella parva</i>	B.G., HU <sup>f</sup>	6-12/3-8	yes	++++
<i>Dunaliella</i> sp. Strain I4	B.G., HU <sup>f</sup>	6-10/3-8	yes	+++
<i>Dunaliella</i> sp. Strain E4	B.G., HU <sup>f</sup>	5-10/3-8	yes	+++
<i>Dunaliella</i> sp. Strain Iran 6	B.G., HU <sup>f</sup>	5-8/3-8	yes	+++
<i>Nannochloris</i> sp. Strain W515	J.L., CUNY	8-12/6-8	no	++
Cyanophyceae				
<i>Anacystis</i> sp.	Houde <sup>g</sup>	2-3	yes	++
Prasinophyceae				
<i>Tetraselmis chuii</i>	IOLR	12-13/8-9	no	-
Prymnesiophyceae				
<i>Isochrysis galbana</i>	IOLR	4-6	yes	++

<sup>a</sup> The dimensions (length/width or diameter) are given in  $\mu\text{m}$ .<sup>b</sup> Rating code: +++++, excellent; +++, good; ++, fair; +, poor; -, no growth.<sup>c</sup> John Lee, City University of New York.<sup>d</sup> Cambridge Culture Collection.<sup>e</sup> Institute of Oceanographic and Limnological Research, Haifa.<sup>f</sup> B. Ginzburg, Hebrew University (Ginzburg and Ginzburg, 1985).<sup>g</sup> E. D. Houde, University of Miami, Florida.

masses of dipterans (Didier and Wilbert, 1976), while *P. magna* was cultivated in cultures to which split peas had been added (Borror, 1972) and presumably fed on the bacterial flora. Nevertheless, comparative dietary experiments on the above-mentioned species should be further extended in order to establish their feeding pattern.



TABLE III

*Chemosensory response of Paranophrys carnivora to different diets as determined by capillary assay*

Diet cultivated on	Test diet offered in capillary		
	<i>Dunaliella parva</i>	<i>Enterobacter aerogenes</i>	<i>Artemia</i> homogenate
Algae ( <i>Dunaliella parva</i> )	—	+	+
Bacteria ( <i>Enterobacter aerogenes</i> )	—	+	+
Fresh meat (wounded <i>Artemia</i> )	—	+	+

Cultures of the ciliates were grown each on a different diet as indicated. The “+” indicates a positive response and “—”, no response.

Our attempts (unpub.) to introduce *P. carnivora* as a symbiont living in the coelenterates *Cordylophora* sp., *Cassiopea* sp., and *Aiptasia* as well as in the crustaceans *Artemia salina* and *Macrobrachium rosenbergii*, did not succeed. Two other species of the genus, *P. marina* and *P. carcini*, were found inside coelenterates (Thompson and

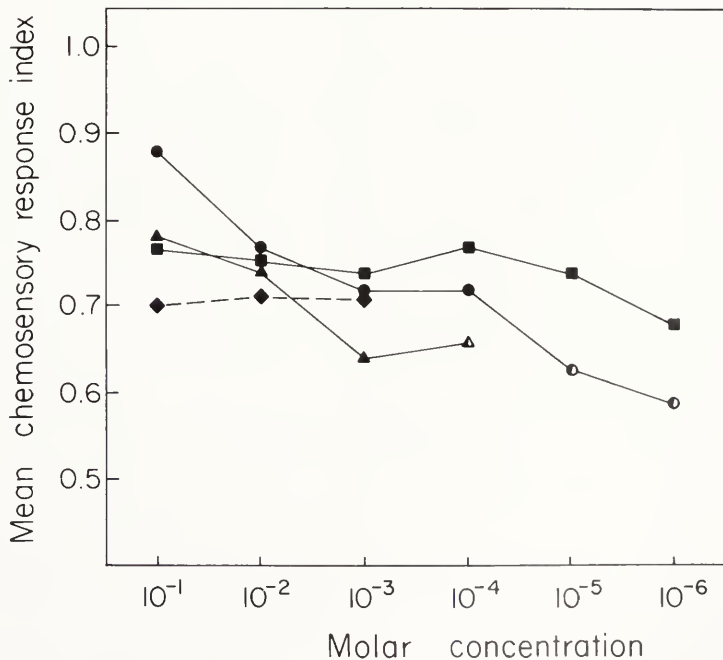


FIGURE 6. Dose-response curves for substances which elicit a positive chemosensory response from *Paranophrys carnivora* by the capillary assay. Each figure represents the mean of the values of the index of chemosensory response obtained from all of the observations for a particular substance at a certain concentration. The results obtained with betaine are indicated by circles, with trimethylamine oxide by triangles, with choline chloride by squares, and with L-histidine by diamonds. A full (black) figure indicates that the mean index of chemosensory response is significantly greater than 0.5 at the 1% level, and a half-full figure, at the 5% level. L-histidine was also tested at 10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup> M, but the results were not significantly greater than 0.5.

Berger, 1965) and in the hemolymph of crustaceans (Grolière and Leglise, 1977), respectively.

Like many other ciliates (Fenchel, 1980a, b, c), *P. carnivora* ingests suspended inert particles such as polystyrene beads (unpub.) and living microorganisms. Here size seems to be a limiting factor in food ingestion. The largest food vacuoles observed did not exceed 7  $\mu\text{m}$  in diameter, and algal species having size ranges above this limit were not ingested *i.e.*, *Amphora* sp., *Dunaliella* strains C9AA, E1, 1644, L10, *Nannochloris* sp., and *Tetraselmis chunii*. However, some of the large species (the four strains of *Dunaliella* mentioned above and *Nannochloris* sp.) did sustain growth. This could be due to the ciliates' feeding on bacteria contaminating the algal cultures and/or on disintegrating algal cells in aged cultures. The same explanation could be offered for the ciliates' growth on *Phaeodactylum tricomutum*. While *C. provasoli* was in the size range of algae that could be engulfed, it was not ingested. This is probably due to the tendency of the latter algal cells to form bigger sized aggregates, or to their having a chemoinhibitory effect on phagocytosis by *Paranophrys*. Those species of algae that were ingested by *Paranophrys* (Table II) gave growth results that varied in their rating from no growth, *i.e.*, *Chlorella saccharophila* and *Dunaliella primolecta*, to excellent growth *i.e.*, *Dunaliella parva* and *Chlorococcum* sp. However, these latter two species did not elicit a positive chemosensory response from *Paranophrys carnivora* in our experiments. Although algae are known to release assimilated carbon into the culture medium (Hunstman, 1972; Fogg, 1977; Saks, 1982), *Dunaliella parva* and *Chlorococcum* sp. evidently do not release a substance eliciting a chemosensory response from *Paranophrys carnivora*.

Betaine, choline, L-histidine, and trimethylamine oxide, the substances found to elicit a positive chemosensory response from *Paranophrys carnivora*, are known to affect feeding behavior in various other organisms (Lindstedt, 1971; Levandowsky and Hauser, 1978; Heinen, 1980; Caprio, 1984). They are also widely distributed in various organisms including bacteria and algae (Bell and Mitchell, 1972; Levandowsky and Hauser, 1978; Edwards, 1982; Galinski and Truper, 1982; Abe, 1983; Konosu *et al.*, 1983; Shirani *et al.*, 1983; Imhoff and Rodriguez-Valera, 1984; Morihiko *et al.*, 1984) and therefore could be indicators of a wide variety of food sources for *Paranophrys carnivora*. There may be other effective substances as yet untested for *Paranophrys carnivora*, which have recently been found to elicit a chemosensory response from other ciliates such as *Paramecium* (Antipa and Norton, 1985) and *Tetrahymena* (Leick and Hellung-Larsen, 1985; Hellung-Larsen *et al.*, 1986). *Paranophrys carnivora* responds to the D-isomer of histidine, which does not occur in nature, and in this respect resembles *Tetrahymena thermophila* (Almagor *et al.*, 1981), which also responds to both the L and D forms of an amino acid (methionine).

Another characteristic *Paranophrys carnivora* shares with *Tetrahymena* is its body transformation. The morphological differences in body proportions between the ovoid feeding form and elongated swimming form of *P. carnivora* appear more pronounced when the ciliate is cultivated on tissues and on rare occasions when grown on algae, after depletion of the food organisms. A similar transformation in form appears after starvation in *Tetrahymena thermophila*. In the latter, the transformation is accompanied by several other changes *i.e.*, oral replacement, caudal cilium appearance, and increase in number of somatic basal bodies and cilia, as well as in speed (Nelsen, 1978; Nelsen and DeBault, 1978). In *P. carnivora*, significant changes in body proportion and an increase in the somatic basal bodies have been noticed. Greater control of culture conditions of the ciliates (as may be obtained with an axenic culture) would enable further discerning and understanding of this phenomenon in *P. carnivora*.

## ACKNOWLEDGMENTS

D. K. gratefully acknowledges the Schonbrunn Foundation for funding the research performed at the Hebrew University of Jerusalem. The assistance of Eli Hatab, M. Devorachek, and A. Nevo in photographing SEM pictures is greatly appreciated. We are grateful to M. Levandowsky for reading the manuscript and making valuable comments.

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